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SERUM ANTIBODI-IMUNOGLOBULIN-G-SUBKLAS SPESIFIK TERHADAP NON-LEUKOTOKSIK *ACTINOBACILLUS ACTINOMYCETEMCOMITANS*

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Abstract

Serum specific IgG subclass antibodies to non-leukotoxic strains of *Actinobacillus actinomycetemcomitans (Aa)* NCTC 9710 and NCTC 10979 were quantified by radioimmunoassay. Samples were taken from sera of 10 patients with Juvenile Periodontitis (JP) and 10 from healthy periodontal individuals as control. The results show that JP patients had higher IgG_2 antibody levels to *Aa. NCTC 9710* (ABT_{50} = 620) than the other IgG subclasses (IgG_1 = 300, IgG_3 = 370, and IgG_4 = 500). The JP patients had also higher IgG_2 antibody levels to *Aa. NCTC 10979* (600) when compared with the other IgG subclasses (IgG_1 =300, IgG_3 = 500, and IgG_4 = 400). Whilst, the control group had lower IgG subclass antibody level as compared with the JP group. In conclusion, the JP patients had an elevation of IgG_2 subclass antibodies to non-leukotoxic *Actinobacillus actinomycetemcomitans*

Abstrak

Serum antibodi Ig-G-subklas spesifik terhadap galur non-leukotoksik-*Acinobacillus actinomycetemcomitans (Aa) NCTC 9710* dan *NCTC 10979* diukur dengan radioimunoasai. Sampel diambil dari serum 10 penderita *Juvenile periodontitis (JP)* dan 10 individu sehat periodontalnya yang dipakai sebagai kontrol. Hasilnya menunjukkan bahwa penderita *JP* mempunyai kadar antibodi IgG_2 terhadap *Aa NCTC 9710*

lebih tinggi (ABT_{50} =620) dibandingkan dengan subklas-IgG lainnya (IgG_1 = 300, IgG_3 =370 dan IgG_4 =560). Penderita *JP* juga mempunyai kadar antibodi subklas IgG_2 lebih tinggi (600) terhadap *Aa NCTC 10979* dibandingkan dengan subklas IgG lainnya (IgG_1 = 300, IgG_3 =500 dan IgG_4 =400). Sedangkan, kelompok kontrol mempunyai kadar subklas IgG yang lebih rendah dibandingkan dengan kelompok *JP*. Kesimpulan, menunjukkan adanya peningkatan subklas IgG_2 terhadap non-leukotoksik *Actinobacillus actinomycetemcomitans* pada penderita *Juvenile Periodontitis*

Introduction

Data from microbiological, histopathological, immunological, and clinical studies indicate that *Actinobacillus actinomycetemcomitans (Aa)* is important in the aetiology of patients with localized juvenile periodontitis (LJP).[1,2,3] *A.actinomycetemcomitans* has been found in high numbers in almost all LJP lesions, whereas in periodontally healthy sites in LJP patients, in normal juveniles, normal adults, or adult periodontitis patients, it is less frequently present and occurs in much lower numbers.[3]

It has been reported that patients with juvenile periodontitis (JP) especially LJP had high antibody levels specific to bacteria *Actinobacillus actinomycetemcomitans (Aa).*[4]

The relative amounts of specific IgG subclass isotypes produced during the antibody response depend on the nature of the antigen. Bacterial protein antigens induce mostly IgG_1 antibodies in humans and low levels of IgG_3 and IgG_4. In contrast, IgG_2 subclass predominates in response to bacterial polysaccharide antigens lipopolysaccharide.[5] In general, antibodies against bacterial cell surface components are beneficial to host defence. The four IgG subclass isotypes (IgG_1, IgG_2, IgG_3, and IgG_4) have various defensive features, including opsonic activity, complement activation and toxin inactivation.[6]

In periodontitis subjects, low avidity antibody and IgG2 antibody, which lacks strong complement fixation and opsonization properties, appear to predominate,[7,8] most likely reducing the effectiveness of the humoral response in clearing pathogens.

In this paper, the level of antibody IgG subclasses specific to *Actinobacillus actinomycetemcomitans* in patients with juvenile periodontitis and healthy controls will be determined by using the radioimmunoassay.

Materials and Methods

Patients

Serum was obtained from 10 patients with *Juvenile Periodontitis* and 10 healthy volunteers. None of the patients presented evidence of diabetes or other systemic disease. All serum samples were stored at -20 C until analysed. The Juvenile periodontitis (JP) group was composed of 10 young patients who had loss of periodontal attachment limited to first molars and incisors, plus up to two additional teeth, with pocket depth > 4 mm (Donaldson). The patients ranged in age from 11 to 29 years with mean age of 19.1.

A control group (C) was chosen on the basis of probing depth measurements of < 3 mm and no evidence of inflammatory periodontal disease other than mild gingivitis.[4] This was composed of 10 subjects ranging in age from 17 to 38 years, with a mean age of 27.8.

Serum Samples

Each serum was aseptically separated from clotted blood after 4 hours storage at 4 C, centrifuged at 900 g for 10 minutes, then aliquoted into sterile tubes and stored at -20 C until analysed.

Bacterial Sonicates

Bacterial sonicates of non-leukotoxic *Actinobacillus actinomycetemcomitans strain NCTC 9710 and NCTC 10979* were obtained from Dawes Soniprobe, London.

Determination of IgG Antibody Subclasses

The radioimmunoassay used for the measurement of antibody titres to bacterial sonicates was based upon solified solid phase radioimmunoassay techniques as described by Smith and Lehner (1981).[9] 100 µl of bacterial sonicates of *Actinobacillus actinomycetemcomitans* at an optimal concentration of 1 µg protein/ml were absorbed into flexible polyvinyl-chloride (PVC) plate (Dynatech Labs.Ltd., Sussex) in triplicate by overnight incubation at room temperature. Subsequently, the unbound antigen was removed, wells were thoroughly washed with PBS, 0.5% bovine serum albumin (BSA) and 0.005% Tween 20 and then blocked by incubation with with PBS, BSA, Tween 20 for 1.5 hours at 37^0 C. After blocking, 50 µl of serum dilution of patients (1:50, 1:250; 1:1250, and 1:6250) were added to each well, incubated for 2 hours at 37^0 C. The plates were washed and then incubated with 50 µl sheep anti human IgG subclasses (MilesLab.Inc.) at a dilution of 1: 50 in buffer for 1 hour at 37^0 C. After washing three times, the coated plates were incubated with 50 µl of ^{125}I radiolabelled rabbit antisheep IgG (30-50 000 cpm) for 1 hour at 37^0 C. The plates were washed three times and bound ^{125}I was counted, converted into relative binding values (100%= binding to human Ig coated wells) according to formula:

$$\text{percentage binding} = \frac{\text{cpm sample - cpm low control}}{\text{cpm high control - cpm low control}} \times 100$$

High control represented wells coated with 10 µl uf human immunoglobulin, and low control represented wells with PBS only. The reference immune and control sera were also included in each experiment. The serum dilutions giving 50% of the *plateau* binding values (ABT_{50}) were then calculated. Statistical analyses of the antibody titres was carried out using the Mann-Whitney test.

Result

The IgG subclasses antibody titres to non-leukotoxic *Aactinomycetemcomitans,* strain NCTC 9710 are summarized in Table 1. The JP group had significantly higher IgG_2 antibody levels (mean ABT_{50} = 620) than the other IgG subclasses (IgG_1=300, IgG_3=370, and IgG_4=560).

The IgG antibody subclasses to *A. actinomycetemcomitans* strain NCTC 10979 are shown also on the Table 1. The JP group also had significantly higher IgG_2 antibody levels (mean 600), when compared to the other IgG antibody subclasses (IgG_1=300, IgG_3 =500, and IgG_4=400). Whilst, control group had lower all IgG subclasses to both non leukotoxic *A. actinomycetemcomitans* strains NCTC 9710 and NCTC 10979 compared with the JP group (Table 1).

Table 1. IgG subclasses of antibodies to bacterial sonicates of nonleukotoxic *A. actinomycetemcomitans* strains NCTC 9710, and NCTC 10979 (ABT_{50} value)

Patient Groups	A. actinomycetemcomitans strain	Serum IgG subclasses			
		G_1	G_2	G_3	G_4
JP	NCTC 9710	300	620*	370	560
C	NCTC 9710	50	32	20	19
JP	NCTC 10979	300	600*	500	400
C	NCTC 10979	33	105	43	115

JP: juvenile periodonitits
C : control
* : the highest

Discussion

This study shows that highest antibody levels of IgG_2 subclass to both strains of bacterial sonicates from nonleukotoxic *A. actinomycetemcomitans* NCTC 9710 and 10979 were found in patients with juvenile periodontitis. IgG_2 is known to bind very weakly to lymphocytes via Fc receptors[10] and to fix complement poorly.[11,12]

Several investigators have described also the subclass distribution of serum IgG anti-bodies against *A. actinomycetemcomitans* in localized juvenile periodontitis patients. The IgG_2 subclass predominates in response to lipopolysaccharide derived from *A actinomy-ctemcomitans.*[13]

Ling et al.[14] also examined the subclass IgG responses to *Aa.* and reported that IgG_2 is hyper-responsive to *Aa* in patients with LJP.

Furthermore, the IgA and IgG subclass isotypes have been compared. Brown et al.[15] have identified the subclass, molecular form, and the elevated level of serum IgA antibody against *Aa.* They indicated that monomeric IgG_1 antibodies to *Aa* sonic extracts predominate in most samples before, during, and after periodontal treatment, suggesting that any protective effects conferred by the IgA response to *Aa* are compromised by proteases derived from this microorganism. Finally, Lu et al.[16] also determined high level of IgG_2 concentration to *Aa* in sera from patients with LJP.

In conclusion, there is an elevation of IgG_2 subclass isotype to bacteria *Actinobacillus actinomycetemcomitans* in patients with Juvenile Periodontitis

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